



MitoCapture™ Apoptosis Detection Kit Cat. No. 475866

Note that this user protocol is not lot-specific and is representative of the current specifications for this product. Please consult the vial label and the certificate of analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions. Full details are available at www.calbiochem.com.

Size

1 kit

Form

100 Tests.

Format

Flow cytometry or fluorescence microscopy

Detection Method

Fluorescence

Storage

Upon arrival, store the entire contents of the kit at -20°C. Following initial thaw, store the Incubation Buffer at 4°C. Avoid freeze/thaw cycles. Protect from light. Following initial thaw, aliquot the MitoCapture™ Reagent and freeze at -20°C. Avoid freeze/thaw cycles.

Intended Use

The Calbiochem® MitoCapture™ Apoptosis Detection Kit provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells by detecting the changes in the mitochondrial membrane potential.

Principle of the Assay

The MitoCapture™ Apoptosis Detection Kit utilizes MitoCapture™, a cationic dye that exhibits distinct fluorescence in healthy cells versus apoptotic cells. In healthy cells, MitoCapture™ reagent accumulates and aggregates in the mitochondria, giving off a bright red fluorescence. In apoptotic cells, MitoCapture™ reagent cannot aggregate in the mitochondria due to the altered mitochondrial membrane potential, and thus remains in the cytoplasm in its monomer form, generating a green fluorescence. The fluorescent signals can be easily detected by fluorescence microscopy using a band-pass filter (used to detect the fluorescent wavelengths of FITC and rhodamine). These signals can also be analyzed by flow cytometry using the Fluorescein Isothiocyanate (FITC) channel for green monomers (Ex/Em = 488/530 ± nm) and the Propidium Iodide (PI) channel for red aggregates (Ex/Em = 488/590 ± nm).

Materials Provided

- **MitoCapture™ Reagent:** 1 bottle, 100 µl

- **Incubation Buffer:** 2 bottles, 100 ml each

Reagent Preparation

• **Incubation Buffer:** aliquot enough Incubation Buffer for the number of assays to be performed (a total of 2 ml for each assay) and pre-warm to 37°C before use.

• **Diluted MitoCapture™ Reagent:** dilute the MitoCapture™ Reagent just prior to use. For each assay, mix 1 µl MitoCapture™ Reagent with 1 ml pre-warmed Incubation Buffer. Vortex to mix.

NOTE: The MitoCapture™ Reagent is poorly soluble in aqueous solutions. To remove particulates (optional), centrifuge the dye solution at 13,000 g for 1 min and carefully transfer the supernatant without disturbing pelleted debris.

Detailed Protocol

Staining with MitoCapture™ Reagent

1. Induce apoptosis in cells by desired methods. Prepare a negative control by incubating a separate flask of cells without any treatment.
2. Following an appropriate incubation time harvest the cells and count. Remove $\sim 1 \times 10^6$ cells for each sample and pellet by centrifugation at 500 g for 5 min.
3. Resuspend the cells in 1 ml Diluted MitoCapture™ Reagent and incubate in a 5% CO₂ incubator at 37°C for 15–20 min.
4. Pellet the cells by centrifugation at 500 g; discard the supernatant.
5. Resuspend in 1 ml of the pre-warmed incubation buffer.
6. Analyze by flow cytometry or fluorescent microscopy (see below).

Detection by Flow Cytometry

If using flow cytometry, analyze cells immediately following step 5. Mitochondria in healthy cells contain MitoCapture™ aggregates that are detectable using the PI channel (usually FL2). MitoCapture™ monomers in apoptotic cells are detectable using the FITC channel (usually FL1), therefore, the cells generating a green fluorescence represent apoptotic cells.

Detection by Fluorescence Microscopy

1. Place the cell suspension from step 5 on a glass slide and cover with a glass coverslip.

NOTE: For analyzing adherent cells, grow cells on a coverslip and perform the entire procedure directly on this coverslip in a culture dish. Following the incubation in step 3, invert coverslip onto a glass slide.

2. Analyze the cells immediately under a fluorescence microscope using a band-pass filter (detects fluorescein and rhodamine). MitoCapture™ reagent that has aggregated in the mitochondria of healthy cells generates a red fluorescence. In apoptotic cells, MitoCapture™ reagent cannot accumulate in the mitochondria and remains in the cytoplasm, generating a green fluorescence.

Toxicity

MSDS available upon request.

Trademarks

Calbiochem® is a registered trademark of EMD Biosciences, Inc.

MitoCapture™ is a trademark of BioVision, Inc.

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